Toxicology in the Fast Lane: Application of High-Throughput Bioassays to Detect Modulation of Key Enzymes and Receptors (Supplemental materials)

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Materials and methods

Chemicals. Most chemicals used in the library were obtained from Chem Service Inc. (West Chester, PA) and Sigma Chemical Co (St Louis, MO). Chemicals were at least 95% pure, and used without further purification. Cyano(6-methoxy-naphthalen-2-yl)methyl *trans*-[(3-phenyloxiran-2-yl)methyl] carbonate (CMNPC), cyano(6-methoxy-2-naphthyl)methyl acetate (CMNA), N-(6-methoxypyridin-3-yl) octanamide (Octanoyl-MP) were prepared previously in the laboratory (Huang et al. 2007; Jones et al. 2005; Shan et al. 2001). 1-Chloro-2,4-dinitrobenzene (CDNB), glutathione and ethoxyresorufin (EROD) were obtained form Sigma-Aldrich. Luciferin H was bought from Promega (Madison, WI). We obtained 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from S. Safe (Texas A&M University, College Station, TX). We purchased dimethyl sulfoxide (DMSO), 17β-estradiol (E_2), and phenol red-free Dulbecco's modified Eagle medium (DMEM) from Sigma Chemical Co. (St. Louis, MO); cell culture reagents and media from Gibco/BRL (Grand Island, NY); and dihydrotestosterone (DHT) from Dr. B. Wilson (UC Davis). We purchased [^{3}H]ryanodine ([^{3}H]Ry, 60–90 Ci/mmol; > 99% pure) from Perkin-Elmer New England Nuclear (Wilmington, DE) and unlabeled Ry (> 99% by ultraviolet-HPLC) from Calbiochem (San Diego, CA). All chemicals and solvents were used without further purification.

Environmental chemicals library. The library was prepared in 2 mL deep well polypropylene 96-well assay blocks (Fisher Scientific, Santa Clara, CA; # 07200700). For every compound, a 1 mL solution at 10 mM in DMSO was prepared in a 2 mL glass vial and the solution was transferred into the assay block using a clean glass syringe. Only compounds totally soluble at 10 mM in DMSO were kept inside the library. In each plate we dispensed 1 mL of DMSO in the first column wells to serve as controls. In the remainder of the plate, we dispensed one compound per well, with 88 compounds total per plate. We created two plates with different

chemicals for a total of 176 compounds. A detail description of the chemical contents in each plate is presented in the supplemental materials. The plates were tightly sealed with EVA copolymer sealing mats (Fisher Scientific #07201112). The plates were then sealed in a 2-mil thick plastic bag, to avoid condensation, and stored at -20°C until use. Upon usage, the plates were let to warm-up at room temperature overnight before to be removed from the plastic bag. Using a robotic pipetting station (Quadra 96 – 96 well automated pipettor; Tomtec, Hamden, CT), each well was first mixed and the compound solutions were diluted 10-fold in DMSO (down to 1 mM) and then in the appropriate buffer and transferred into 96 well plates.

Enzyme-based assays.

Hydrolases and GSTs. The sEH activity was measured following the method of Jones et al. 2005; the CESs and PON2 activities were measured following the method of Shan and Hammock, 2001; the FAAH activity was measured following the method of Huang et al. 2007; and the liver cytosolic activity was measured following the method of Habig et al. 1974. For the assay 96-well plates containing 20μL of 10x concentrated test-compound solutions, 150 μL of the appropriate buffer were added in wells A1 to D1 (these four wells served as background control, while wells E1 to H1 served as full activity control), and 150 μL of the enzyme diluted in the same buffer were added to the rest of the plate using our Miniprep robotic system (Tecan, Durham, NC),. The plate was then mixed and incubated at 30 °C for 5 minutes. Across the plate, 30 μL of the working substrate solution (267 μL of 100x substrate solution in DMSO or ethanol diluted with 3,763 μL of buffer) were added quickly to yield the concentration of substrate given in Table 1. The activity was immediately measured at 30 °C kinetically for 10 min in a Spectramax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA) in either fluorescent or absorbance mode using the published optimal wavelength for each substrate.

P450 1A2 & 2C6. Microsomal 7-ethoxyresorufin dealkylation activity (EROD) was

measured following a modified method described by Dutton and Parkinson (1989). To the back plates containing 20 µL of the 10x inhibitor dilution, 160 µL of the human liver microsomal preparation diluted in buffer were added across the plate, except in wells A1 to D1 that received 160 µL of buffer only (these wells served as background control). Using a repeating syringe, 2 µL of 100x EROD solution in DMSO were added to each well. The plate was then mixed and incubated at 30°C for 5 minutes. The enzymatic reaction was started by the addition across the plate of 20 µL of NADPH generating system (Watanabe and Hammock 2001). The resorufin formed was detected fluorometrically ($\lambda_{ex.}$ 535 mn; $\lambda_{em.}$ 585 nm) for 30 min at 30°C in a Spectramax M2 fluorometer.

P450 2C9. The Luciferin-H activity was performed following the method described by (Cali et al. 2006). To the white plate containing 20 µL of the 10x inhibitor dilution, 160 µL of the human liver microsomal preparation diluted in buffer were added across the plate, except in wells A1 to D1 that received 160 µL of buffer only (these wells served as background control). Using a repeating syringe, 2 µL of 100x luciferin-H solution in DMSO were added to each well. The plate was then mixed and incubated at 30°C for 5 minutes. The enzymatic reaction was started by the addition across the plate of 20 µL of NADPH generating system (Watanabe and Hammock 2001). The plates were mixed and incubated at 30°C for 30 minutes. The reaction was stopped and the produced luciferin was revealed by adding 100 µL of luciferase solution provided in the kit from Promega. After 15 minutes at 30°C, the luminescence was measured on a Spectrafluor plus lumimeter (Tecan).

Cell-based bioassay.

Aryl hydrocarbon Receptor (AhR) bioassay. Recombinant mouse hepatoma (H1L6.1c2) cells were grown and maintained as previously described (Garrison et al. 1996; Han et al. 2004). These cells contain the stably integrated, dioxin-responsive–element (DRE)-driven firefly

luciferase reporter gene plasmid pGudLuc6.1. Transcriptional activation of the plasmid occurs in a ligand-, dose-, time- and AhR-dependent manner. Cells were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and allowed to attach for 24 hr. Cells were incubated with carrier solvent DMSO (1% final solvent concentration), TCDD (1 nM), or the indicated compound (10 μM) for 24 hr at 37°C. For luciferase measurement, sample wells were washed twice with phosphate-buffered saline, followed by addition of cell lysis buffer (Promega, Madison, WI); the plates were then shaken for 20 min at room temperature to allow cell lysis. We measured luciferase activity in each well using a Orion microplate luminometer (Berthold, Oak Ridge, TN) with automatic injection of Promega stabilized luciferase reagent. Luciferase activity in each well was expressed relative to that maximally induced by TCDD.

Androgen Receptor (AR) bioassays. For the cell-based human AR-responsive bioassay, recombinant human cells [T47D-androgen-responsive element (ARE)] were grown and maintained as described above for H1L6.1c2 cells. The T47D-ARE cells contain a stably integrated AR-responsive firefly luciferase reporter gene plasmid, pGudLuc7ARE (Rogers and Denison 2000). Cells were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and allowed to attach for 24 hr. Cells were incubated with carrier solvent (DMSO; 1% final solvent concentration), dihydrotestosterone (DHT, 10 nM), or the indicated compound (10 μM) for 24 hr at 37°C. Luciferase activity was measured as described above and activity in each well expressed relative to that maximally induced by DHT.

Estrogen Receptor (ER) bioassay. Recombinant human ovarian cancer cells (BG1Luc4E$_2$, ER-α–positive) were grown and maintained as previously described (Rogers and Denison 2000). These cells contain a stably integrated, ER-responsive firefly luciferase reporter plasmid, pGudLuc7ERE. Cells were maintained in estrogen-stripped media for 5 days before they were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and

allowed to attach for 24 hr. Cells were then incubated with carrier solvent (DMSO: 1% final solvent concentration), 17β-estradiol (E_2, 1 nM), or the indicated compound (10 μM) for 24 hr at 37°C. Luciferase activity was measured as described above and activity expressed relative to that maximally induced by E_2.

Ryanodine receptor 1 and 2 (RyR1 and RyR2) bioassay. Sarcoplasmic reticulum (SR) membrane vesicles enriched in ryanodine receptor (RyR1) were prepared from back and hind limb skeletal muscles of New Zealand White rabbits according to the method of Saito et al. (1984). Heavy SR enriched in RyR2 from rat cardiac ventricles was prepared by sucrose-density gradient centrifugation, as described previously by Pessah et al. (1990). The preparations were stored in 10% sucrose, and 5 mM imidazole (pH 7.4) at -80°C until needed. Equilibrium of specific high-affinity [^{3}H]ryanodine ([^{3}H]Ry) binding were determined according to the method of Pessah et al. (1987). [^{3}H]Ry binds with high affinity and specificity to the open state of RyR1 and RyR2 and therefore provides a convenient measure of ligands that influence channel conformation (Pessah et al. 1985 and 1987; Zimanyi et al. 1991). SR vesicles enriched with RyR1 (50 μg protein/ml) or RyR2 (100 μg protein/mL) were incubated with a compound (5 μM, and its solvent dimethyl sulfoxide (DMSO) served for control) in assay buffer containing HEPES (20 mM, pH 7.4), KCl (250 mM), NaCl (15 mM), [^{3}H]Ry (2 nM) and $CaCl_2$ (20μM, adjusted with EGTA; Brooks and Storey, 1992). Nonspecific binding was determined by incubating SR with 1000-fold excess unlabeled ryanodine in the absence or presence of the compound. The binding reactions were kept in 37°C for 3hr and then quenched by filtration through GF/B glass fiber filters and washed twice with ice-cold harvest buffer (20 mM Tris-HCl, 250 mM KCl, 15 mM NaCl, and 20 μM $CaCl_2$, pH 7.4). Total n = 8 samples/compound or DMSO from two independent measurements under the identical conditions were taken for data analysis.

References

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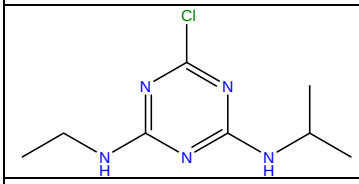
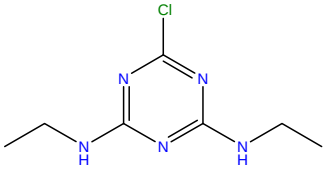
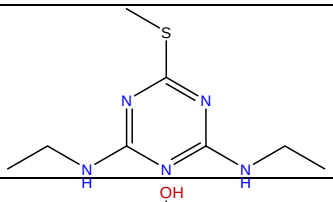
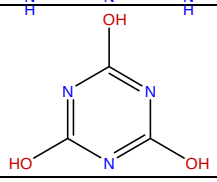
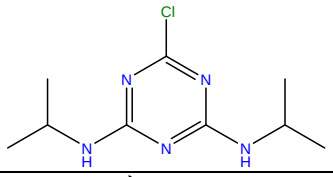
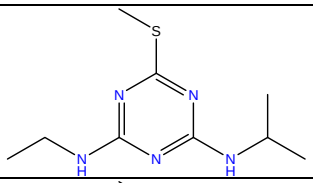
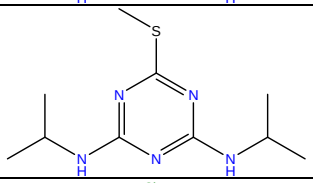
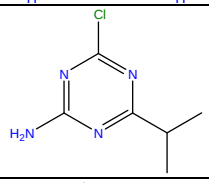
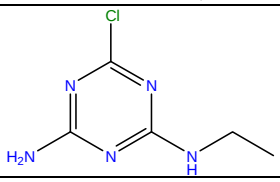
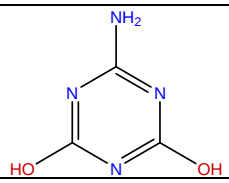
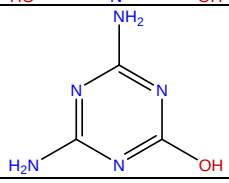
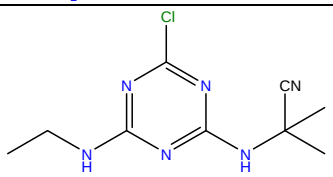
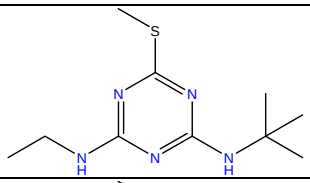
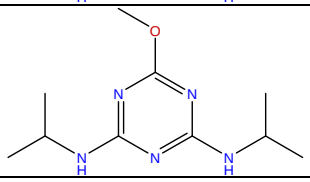
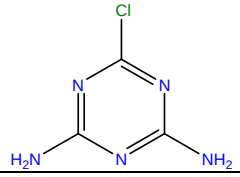
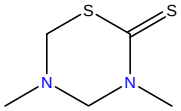
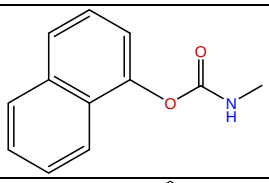
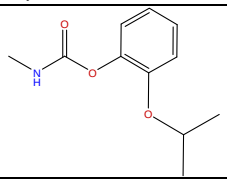
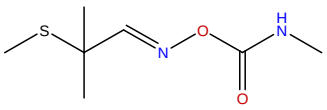
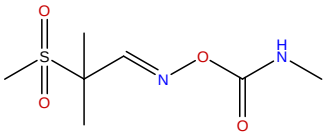
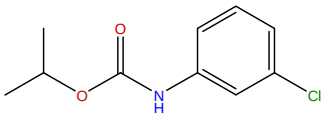
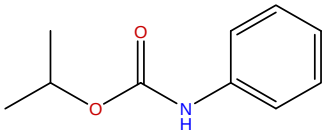
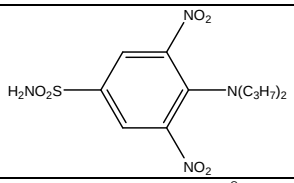
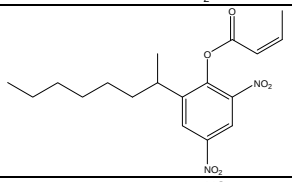
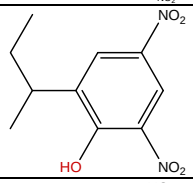
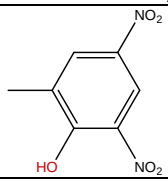
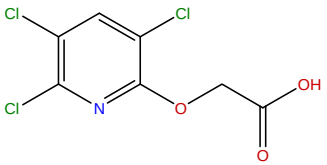
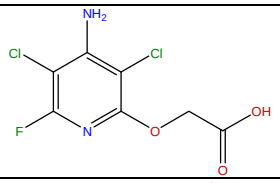
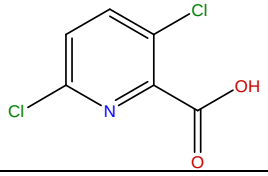
Supplemental Material, Table 1. Overall composition of library of chemicals tested.

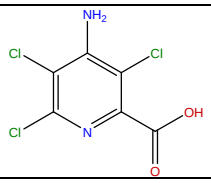
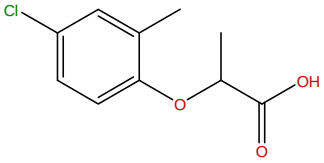
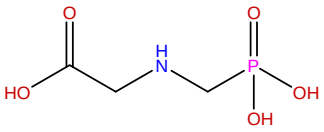
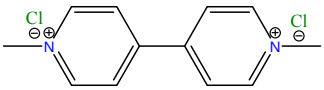
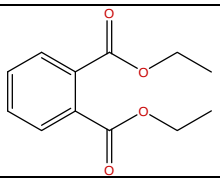
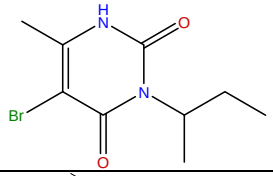
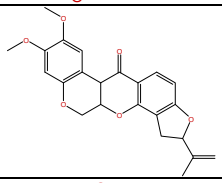
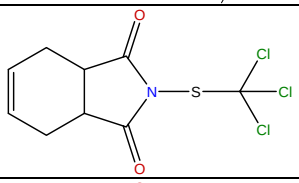
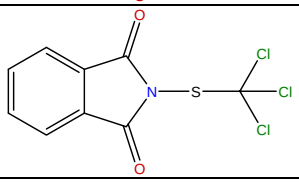
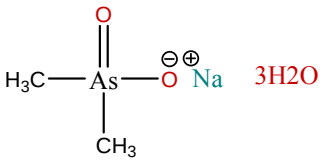
Pesticides (155)		Industrial products (32)	
Fungicide	20	Detergent	4
Herbicide	63	Exhaust pollutant	4
Insecticide	63	Flame retardant	5
Metabolite	4	Food additive	2
Microbiocide	3	Pharmaceutical drug	5
Nematocide	1	Plant growth regulator	9
Piscicide	1	Plastic product	3

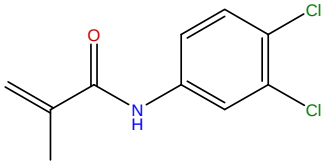
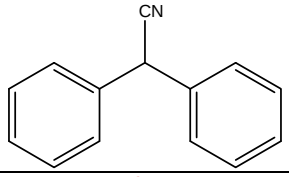
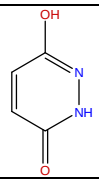
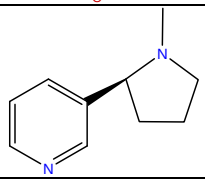
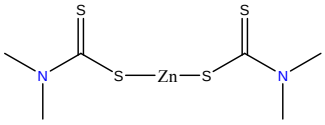
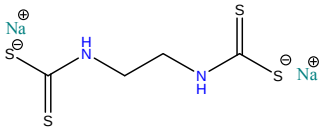
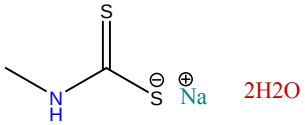
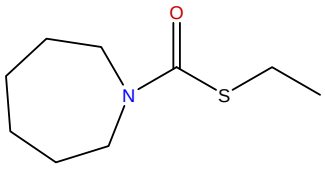
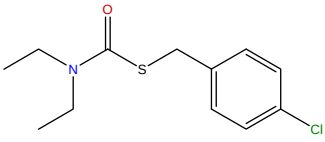
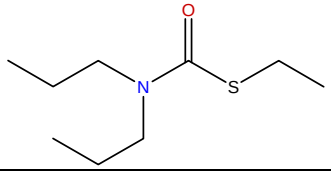
Supplemental Material, Table 2. Detail composition of the library of compounds used.

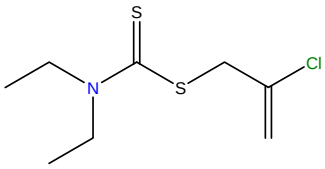
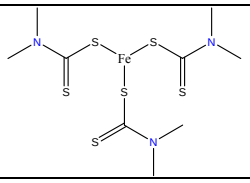
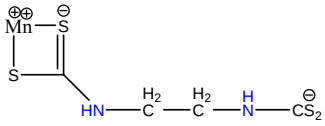
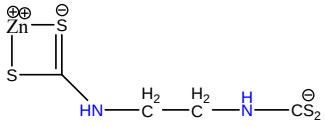
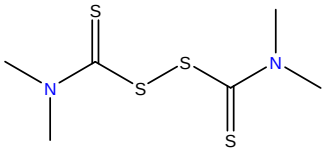
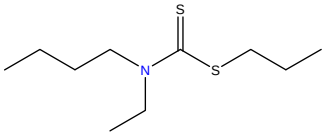
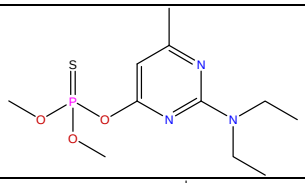
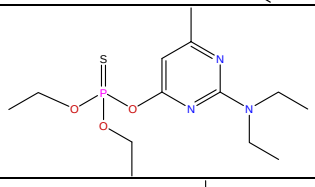
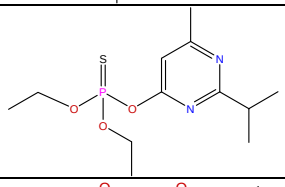
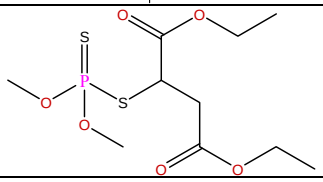
Structure	#	Name	Plate	Row	Column	Usage
	P1	Atrazine	I	A	2	Herbicide
	P2	Simazine	I	B	2	Herbicide
	P3	Simetryn	I	C	2	Herbicide
	P4	Cyanuric acid	I	D	2	Herbicide
	P5	Propazine	I	E	2	Herbicide
	P6	Ametryn	I	F	2	Herbicide
	P7	Prometryn	I	G	2	Herbicide
	P8	2-Chloro-4-isopropyl-6-amino-s-triazine	I	H	2	Herbicide
	P9	2-Chloro-4-ethylamino-6-amino-s-triazine	I	A	3	Herbicide

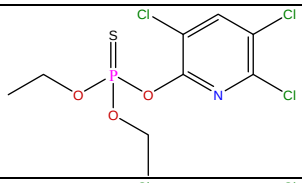
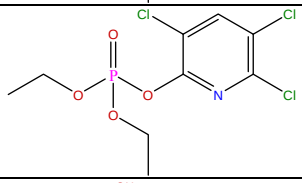
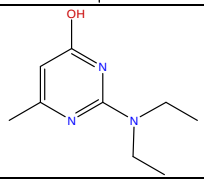
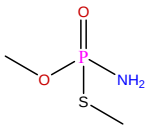
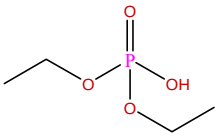
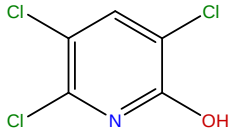
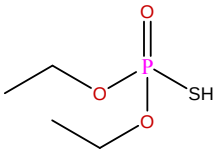
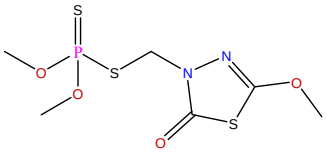
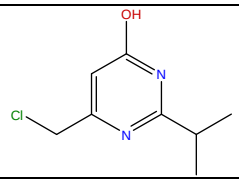
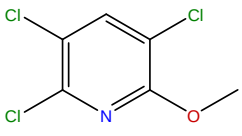
	P10	Ammelide	I	B	3	Herbicide
	P11	Ammeline	I	C	3	Herbicide
	P12	Cyanazine	I	D	3	Herbicide
	P13	Terbutryn	I	E	3	Herbicide
	P14	Prometon	I	F	3	Herbicide
	P15	2-Chloro-4,6-diamino-s-triazine	I	G	3	Herbicide
	P16	Dazomet	I	H	3	Fungicide
	P17	Carbaryl	I	A	4	Insecticide
	P18	Propoxur	I	B	4	Insecticide
	P19	Aldicarb	I	C	4	Insecticide

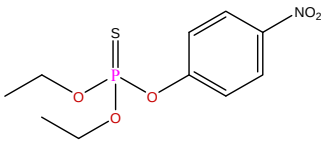
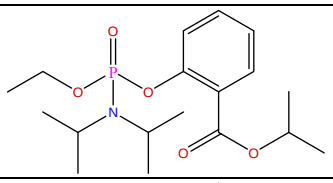
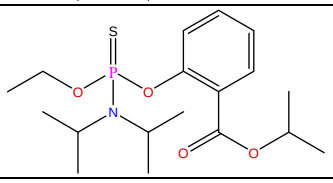
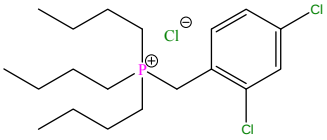
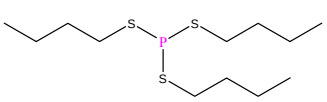
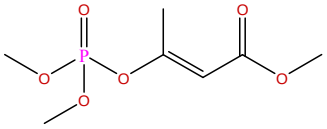
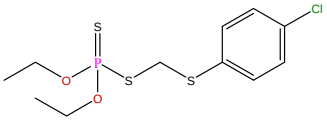
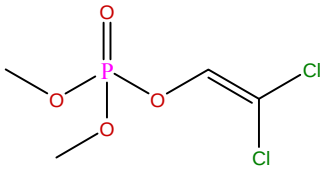
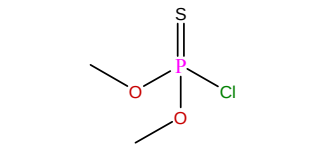
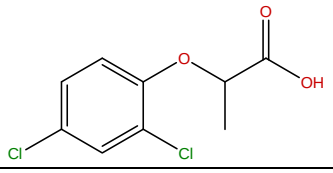
	P20	Aldoxycarb	I	D	4	Insecticide Nematocide
	P21	Isopropyl-N-[m-chlorophenyl] carbamate	I	E	4	Herbicide
	P22	Isopropyl-N-phenylcarbamate	I	F	4	Herbicide
	P23	Oryzalin	I	G	4	Herbicide
	P24	2-methylheptyl-4,6-dinitrophenyl Crotonate	I	H	4	Fungicide Acramicide
	P25	DNBP	I	A	5	Herbicide
	P26	4.6-Dinitro-o-cresol	I	B	5	Fungicide Insecticide Herbicide
	P27	Triclopyr	I	C	5	Herbicide
	P28	Fluroxypyr	I	D	5	Herbicide
	P29	Clopyralid	I	E	5	Herbicide

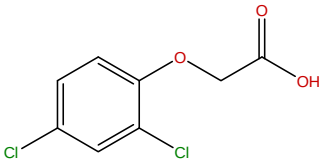
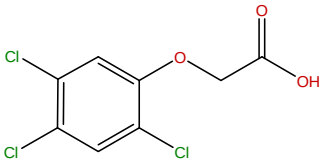
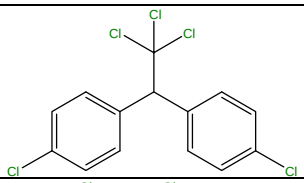
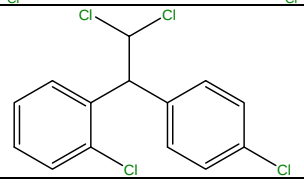
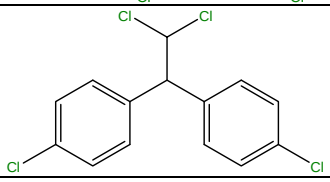
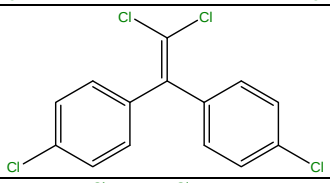
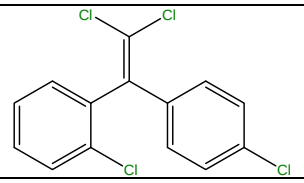
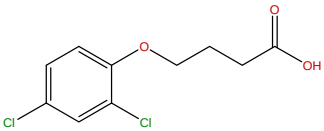
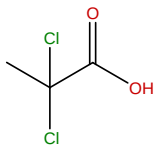
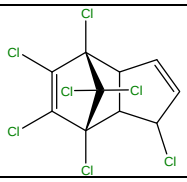
	P30	Picloram	I	F	5	Herbicide
	P31	Mecoprop	I	G	5	Herbicide
	P32	Glyphosate	I	H	5	Herbicide
	P33	Paraquat dichloride	I	A	6	Herbicide
	P34	Diethyl phthalate	I	B	6	Plasticizer
	P35	Bromacil	I	C	6	Herbicide
	P36	Rotenone	I	D	6	Insecticide Piscicide
	P37	Captan	I	E	6	Fungicide
	P38	Folpet	I	F	6	Fungicide
	P39	Cacodylic acid, Na salt	I	G	6	Herbicide

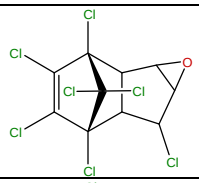
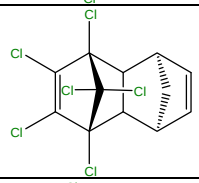
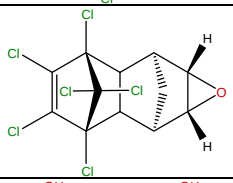
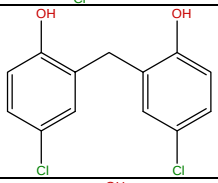
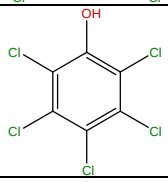
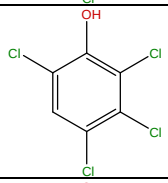
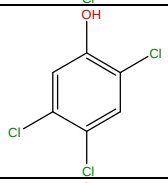
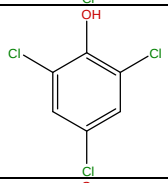
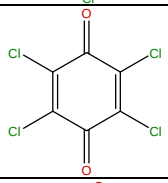
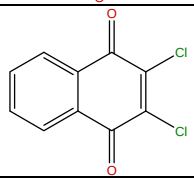
	P40	Chloranocryl	I	H	6	Herbicide
	P41	Diphenyl acetonitrile	I	A	7	Pesticide
	P42	Maleic acid hydrazide	I	B	7	Herbicide
	P43	Nicotine	I	C	7	Insecticide
	P44	Ziram	I	D	7	Fungicide
	P45	Nabam	I	E	7	Fungicide
	P46	Metam sodium	I	F	7	Fungicide Herbicide
	P47	Molinate	I	G	7	Herbicide
	P48	Thiobencarb	I	H	7	Herbicide
	P49	Eptam	I	A	8	Fungicide

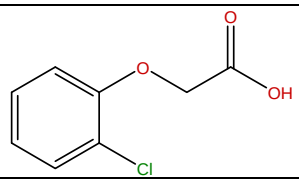
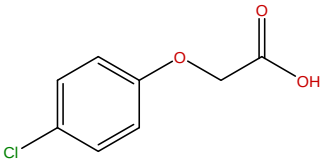
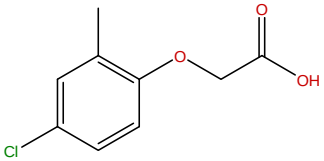
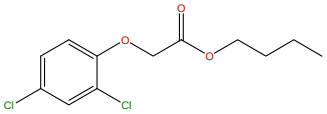
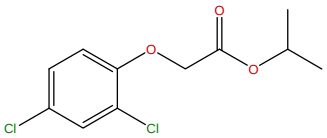
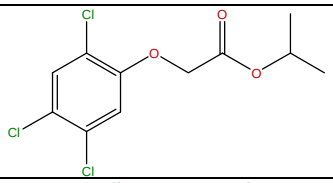
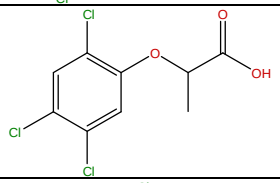
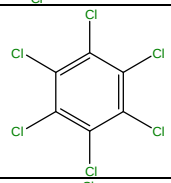
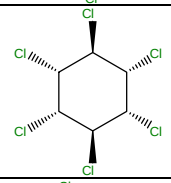
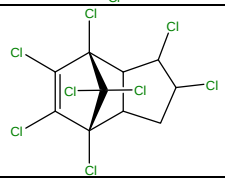
	P50	CDEC	I	B	8	Fungicide
	P51	Ferbam	I	C	8	Fungicide
	P52	Maneb	I	D	8	Fungicide
	P53	Zineb	I	E	8	Fungicide
	P54	Tetramethyl-thiuram disulfide	I	F	8	Fungicide
	P55	S-propyl butylethyl-thiocarbamate	I	G	8	Herbicide
	P56	Pirimiphos – methyl	I	H	8	Insecticide
	P57	Pirimiphos – ethyl	I	A	9	Insecticide
	P58	Diazinon	I	B	9	Insecticide
	P59	Malathion	I	C	9	Insecticide

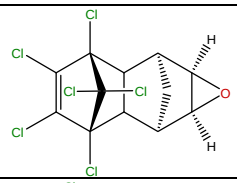
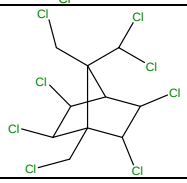
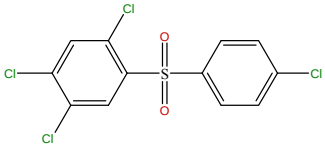
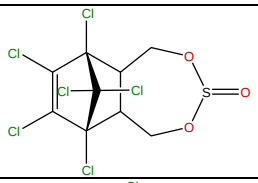
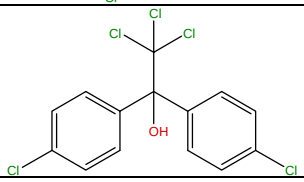
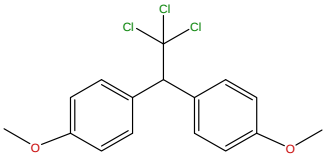
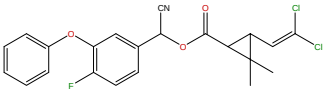
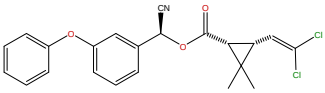
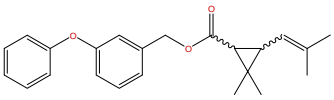
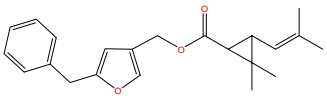
	P60	Chlorpyrifos	I	D	9	Insecticide
	P61	Chlorpyrifos oxon	I	E	9	Insecticide
	P62	2-diethylamino-6-methylpyrimidin-4-ol	I	F	9	Insecticide metabolite
	P63	Methamidophos	I	G	9	Insecticide
	P64	Diethyl phosphate	I	H	9	Insecticide
	P65	3,5,6-Trichloro-2-pyridinol	I	A	10	Insecticide metabolite
	P66	O,O-Diethylthio-phosphate	I	B	10	Insecticide
	P67	Methidathion	I	C	10	Insecticide
	P68	6-Chloromethyl-4-hydroxy-2-isopropyl pyrimidine	I	D	10	Insecticide metabolite
	P69	2-Methoxy-3,5,6-trichloropyridine	I	E	10	Insecticide metabolite

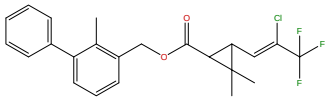
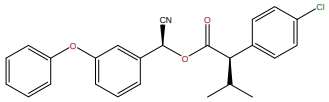
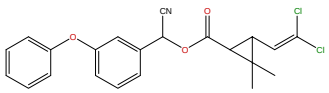
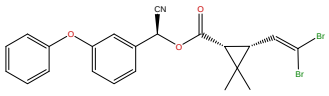
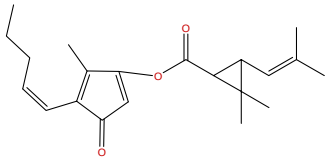
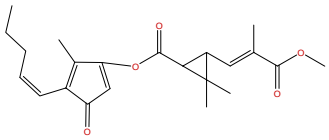
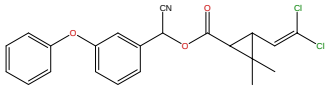
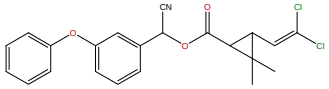
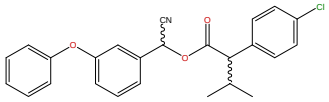
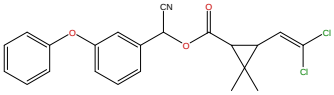
	P70	Parathion	I	F	10	Insecticide
	P71	des-N-Isopropyl isophenphos oxygen analog	I	G	10	Insecticide
	P72	des-N-Isopropyl isophenphos	I	H	10	Insecticide
	P73	Tributyl (2,4-dichlorobenzyl)-phosphonium chloride	I	A	11	Herbicide
	P74	Tributyl phosphoro-trithioite	I	B	11	Herbicide
	P75	Phosdrin	I	C	11	Insecticide
	P76	Carbophenothion	I	D	11	Insecticide
	P77	DDVP	I	E	11	Insecticide
	P78	O,O-dimethyl phosphochloridothioate	I	F	11	Herbicide
	P79	Dichlorprop	I	G	11	Herbicide

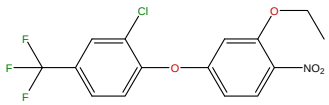
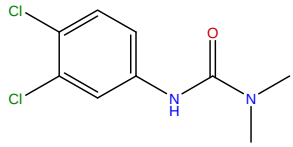
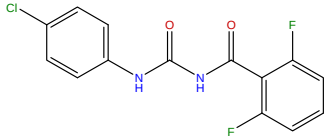
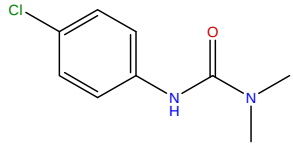
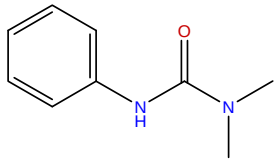
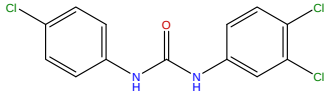
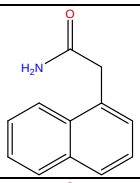
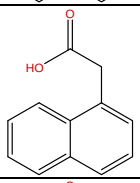
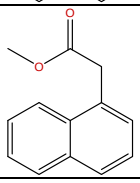
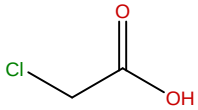
	P80	2,4-D	I	H	11	Herbicide
	P81	2,4,5-T	I	A	12	Herbicide
	P82	p,p-DDT	I	B	12	Insecticide
	P83	o,p'-DDD	I	C	12	Insecticide
	P84	p,p-DDD	I	D	12	Insecticide
	P85	p,p-DDE	I	E	12	Insecticide
	P86	o,p'-DDE	I	F	12	Insecticide
	P87	2,4-DB	I	G	12	Herbicide
	P88	Dalapon	I	H	12	Herbicide
	P89	Heptachlor	II	A	2	Insecticide

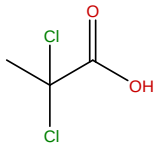
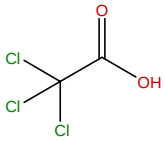
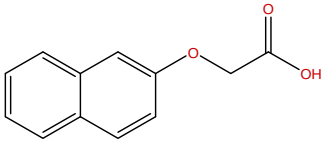
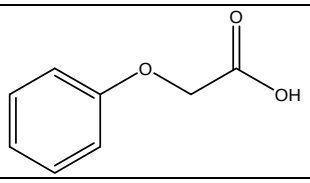
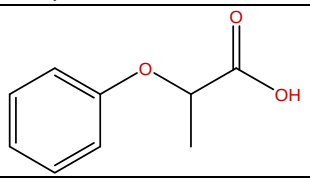
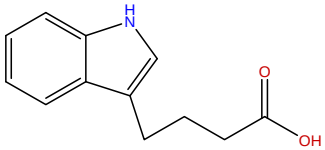
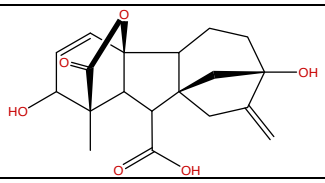
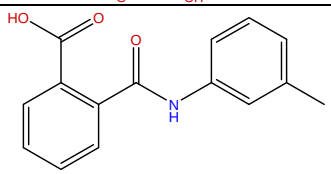
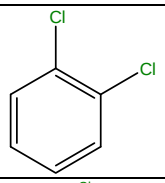
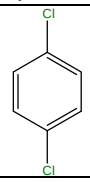
	P90	Heptachlor epoxide	II	B	2	Insecticide
	P91	Aldrin	II	C	2	Insecticide
	P92	Dieldrin	II	D	2	Insecticide
	P93	2,2' Methylenebis(4-chlorophenol)	II	E	2	Microbiocide
	P94	Pentachlorophenol	II	F	2	Fungicide Herbicide Insecticide
	P95	2,3,4,6-Tetrachlorophenol	II	G	2	Herbicide
	P96	2,4,5-Trichlorophenol	II	H	2	Herbicide
	P97	2,4,6-Trichlorophenol	II	A	3	Fungicide Herbicide Insecticide
	P98	Chloranil	II	B	3	Fungicide
	P99	Dichlone	II	C	3	Fungicide

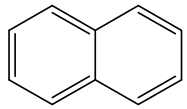
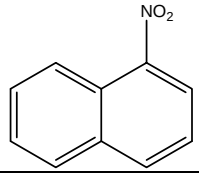
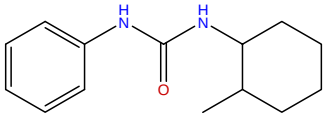
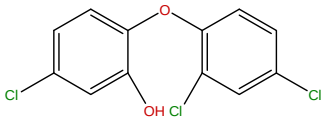
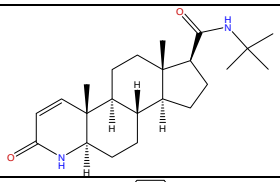
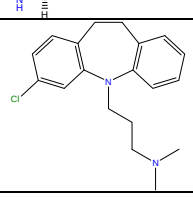
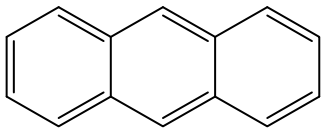
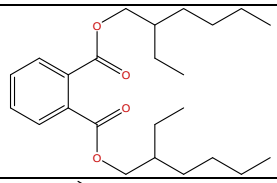
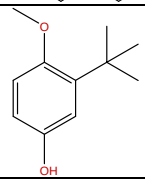
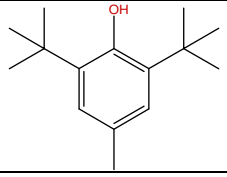
	P100	o-Chlorophenoxy acetic acid	II	D	3	Herbicide
	P101	p-Chlorophenoxy acetic acid	II	E	3	Herbicide
	P102	MCPA	II	F	3	Herbicide
	P103	2,4-Dichlorophenoxy acetic acid, butyl ester	II	G	3	Herbicide
	P104	2,4-Dichlorophenoxy acetic acid, isopropyl ester	II	H	3	Herbicide
	P105	2,4,5-Trichlorophenoxy acetic acid, isopropyl ester	II	A	4	Herbicide
	P106	Silvex	II	B	4	Herbicide
	P107	Benzene hexachloride	II	D	4	Insecticide
	P108	Lindane	II	E	4	Insecticide
	P109	Chlorodane	II	F	4	Insecticide

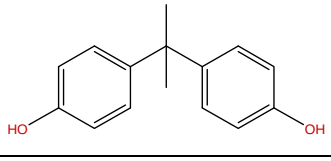
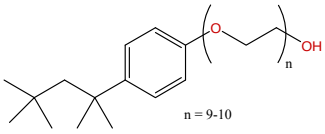
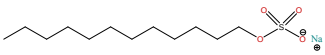
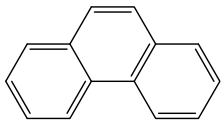
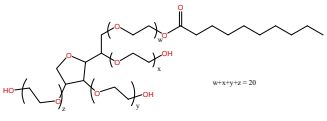
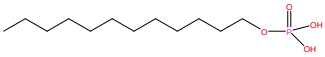
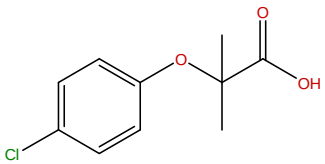
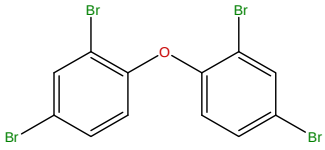
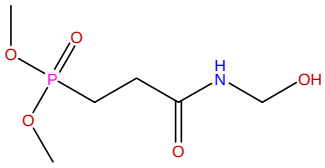
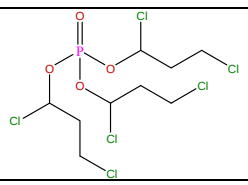
	P110	Endrin	II	G	4	Insecticide
	P111	Toxaphene	II	H	4	Insecticide
	P112	Tedion	II	C	4	Insecticide
	P113	Thiodan	II	A	5	Insecticide
	P114	4,4'-Dichloro-a-(trichloromethyl)-benzhydrol	II	B	5	Insecticide
	P115	Methoxychlor	II	C	5	Insecticide
	P116	Baythroid	II	D	5	Insecticide
	P117	a-Cypermethrin	II	E	5	Insecticide
	P118	d-(*cis*/*trans*) Phenothrin	II	F	5	Insecticide
	P119	Resmethrin	II	G	5	Insecticide

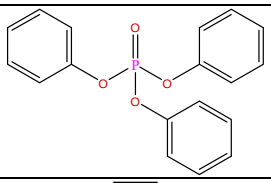
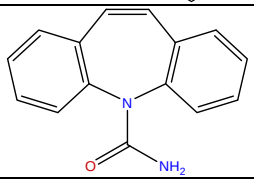
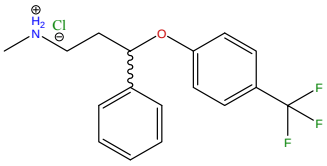
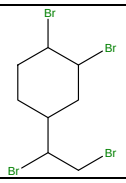
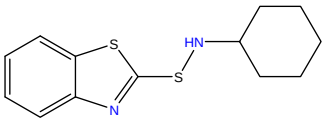
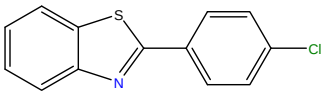
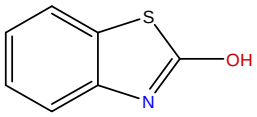
	P120	Bifenthrin	II	H	5	Insecticide
	P121	Asana	II	A	6	Insecticide
	P122	*zeta*-Cypermethrin	II	B	6	Insecticide
	P123	Deltamethrin	II	C	6	Insecticide
	P124 A	Pyrethrum	II	D	6	Insecticide
	P124 B	Pyrethrum	II	E	6	Insecticide
	P125	Cypermethrin (mix of isomers)	II	F	6	Insecticide
	P126	*trans*-Cypermethrin	II	G	6	Insecticide
	P127	Sanmarton	II	H	6	Insecticide
	P128	*cis*-Cypermethrin	II	A	7	Insecticide

	P129	Oxyfluorfen	II	B	7	Herbicide
	P130	Diuron	II	C	7	Herbicide
	P131	Diflubenzuron	II	D	7	Insecticide
	P132	Monuron	II	E	7	Herbicide
	P133	Fenuron	II	F	7	Herbicide
	P134	3,4,4'-Trichloro-carbanilide (Trichlocarban)	II	G	7	Microbiocide
	P135	1-Naphthalene acetamide	II	H	7	Plant growth Regulator
	P136	1-Naphthalene acetic acid	II	A	8	Plant growth Regulator
	P137	1-Naphthaleneacetic acid, methyl ester	II	B	8	Plant growth Regulator
	P138	Chloracetic acid	II	C	8	Herbicide

	P139	2,2-Dichloropropionic acid	II	D	8	Herbicide
	P140	Trichloroacetic acid	II	E	8	Herbicide
	P141	2-Naphthoxyacetic acid	II	F	8	Plant Growth Regulator
	P142	Phenoxyacetic acid	II	G	8	Plant Growth Regulator
	P143	2-Phenoxy propionic acid	II	H	8	Plant Growth Regulator
	P144	3-Indolebutyric acid	II	A	9	Plant Growth Regulator
	P145	Gibberellic acid	II	B	9	Plant Growth Regulator
	P146	N-*m*-Tolyl-phthalamic acid	II	C	9	Plant Growth Regulator
	P147	*o*-Dichloro-benzene	II	D	9	Insecticide
	P148	*p*-Dichloro-benzene	II	E	9	Insecticide

	P149	Naphthalene	II	F	9	Insecticide Exhaust pollutant
	P150	1-Nitro-naphthalene	II	G	9	Exhaust pollutant
	P151	Siduron	II	H	9	Herbicide
	P152	Irgasan (Triclosan)	II	A	10	Microbiocide
	P153	Finasteride	II	B	10	Anti-androgen
	P154	Clomipramine	II	C	10	Anti-depressant
	P155	Anthracene	II	D	10	Insecticide Exhaust pollutant
	P156	DEHP	II	E	10	Plasticizer
	P157	BHA	II	F	10	Food additive
	P158	BHT	II	G	10	Food additive

	P159	Bisphenol A	II	H	10	Plastic monomer
	P160	Triton X-100	II	A	11	Detergent
	P161	SDS	II	B	11	Detergent
	P162	Phenanthrene	II	C	11	Exhaust pollutant
	P162	Tween - 20	II	D	11	Detergent
	P163	n-Dodecyl phosphoric acid	II	E	11	Detergent
	P164	Clofibric acid	II	F	11	Lipid regulator
	P165	PBDE-47	II	G	11	Flame retardant
	P166	Pyrovatex CP	II	H	11	Flame retardant
	P167	Amgard CJ	II	A	12	Flame retardant

	P168	Triphenyl phosphate	II	B	12	Flame retardant Plasticizer
	P169	Carbamazepine	II	C	12	Anti-convulsant
	P170	Fluoxetine HCl	II	D	12	Anti-depressant
	P171	1,2-Dibromo-4-(1,2-dibromo-ethyl)cyclohexane	II	E	12	Flame retardant
	P172	N-Cyclohexyl-2-benzothiazyl sulfenamide	II	F	12	Fungicide
	P173	2-(4-Chloro-phenyl)-benzothiazole	II	G	12	Fungicide
	P174	2-Hydroxy-benzothiazole	II	H	12	Fungicide

Supplemental Material, Figure 1: Positive hits map from primary screening of 9 enzymes activities and 5 receptors bioassay.

Plate I	2	3	4	5	6	7	8	9	10	11	12
A			1	1				1			
B				1						1	
C					1		1			1	1
D				1	1		2	2		3	
E							1	1			
F							2		1		2
G											
H							2				

Plate II	2	3	4	5	6	7	8	9	10	11	12
A					1				4		
B		1							2		4
C		2									
D			1	1	2						
E	1			1	1				1		
F	1		1								1
G						1					1
H								1	1		

Supplemental Material, Figure 2: Determination of the K_I of triclosan with the Human CES1 Using CMNA as Substrate. For each substrate concentration (5 to 100 μM), the velocity is plotted as a function of triclosan concentration (0 to 1000 nM), allowing the determination of an apparent inhibition constant (K_{Iapp}). K_{Iapp}s are plotted as a function of the substrate concentration (insert). For [S] = 0, a K_I value of 103 nM was found.